

DECOMPOSITION AND REGENERATION OF NITROGENOUS ORGANIC MATTER IN SEA WATER

IV. INTERRELATIONSHIP OF VARIOUS STAGES; INFLUENCE OF CONCENTRATION AND NATURE OF PARTICULATE MATTER¹

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In previous investigations (1937, 1939, 1940) it has been demonstrated that the decomposition of particulate organic matter in sea water proceeds in well-defined steps, the main stages being the formation of ammonia, nitrite and nitrate. The appearance of these substances is due to the successive development of different bacterial floras acting upon the original substratum of organic matter. In nature, however, a mixture of the various floras will probably occur, with frequent or continuous addition of new decomposing material. It seemed desirable, therefore, to study the interrelationship of the different stages of the cycle and the results obtained when two or more stages occur simultaneously.

For this purpose a 20-liter carboy of filtered sea water from Woods Hole Harbor, to which washed diatoms (*Nitzschia Closterium*) were added, was kept at room temperature in the dark. In order to determine what effect the bacterial flora present at various stages would have on the decomposition of fresh organic matter, portions of the culture were withdrawn at various times during the decomposition cycle. New particulate organic matter was added to these subcultures, as indicated below, after which they were put in fresh containers in the dark. The parent culture (No. 42) and the various subcultures (42A to 42H) were analyzed regularly for the different forms of nitrogen and the changes found are shown in Fig. 1.

The first subculture, No. 42A, was separated from the parent culture when the ammonia in the latter had reached its maximum and when the first trace of nitrite had appeared; the second subculture (42B) a few days later, when the nitrite formation was well under way; and the third (42C), when the nitrite had about reached its maximum. Later, when the nitrite began to decline in subculture No. 42B and when nitrate formation had begun, a portion of it was used in preparing a new subculture (42G). To all these subcultures new particulate matter was added in the form of living, washed diatoms.

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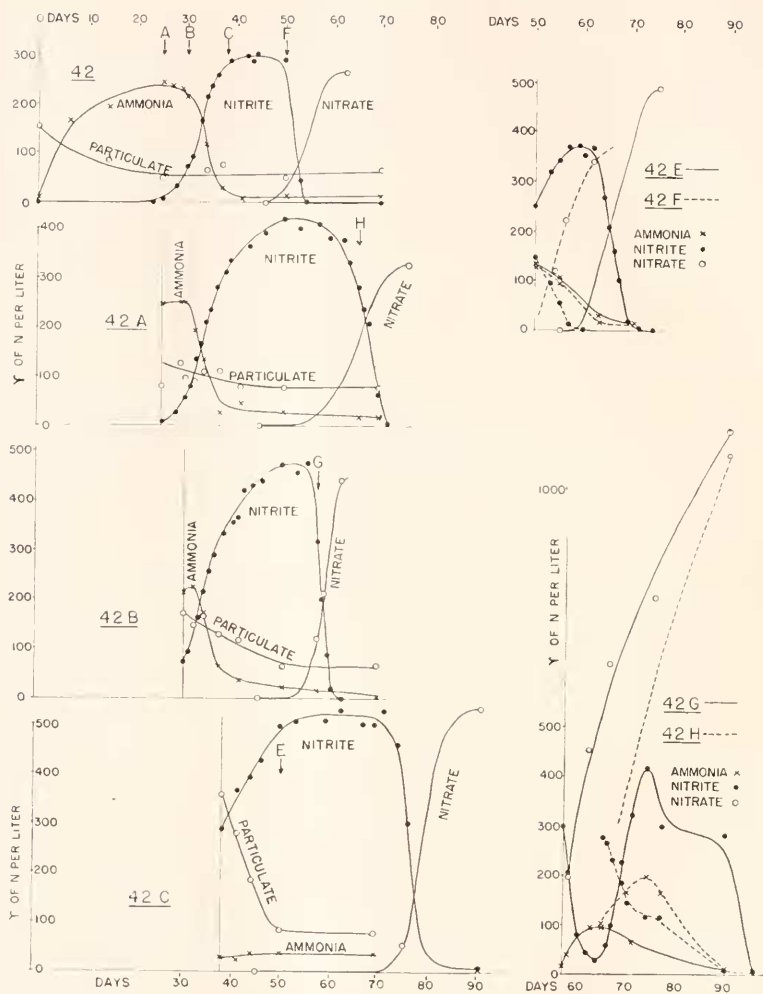


FIG. 1. Interrelationship of different stages of the decomposition cycle. Time in days. Different forms of nitrogen in micrograms (γ) per liter. The original culture, No. 42, consisted of filtered sea water with washed diatoms (*Nitzschia Closterium*) added. Decomposition in the dark. Subcultures A, B, etc., separated at times indicated by arrows and with new particulate organic matter added.

Figure 1 shows that nitrite formation in the first three subcultures (42A, B and C) was in no way interfered with by the addition of new diatoms, but proceeded at normal speed without interruption. In each case ammonia rose only slightly higher than in the original culture, No.

42, indicating that the ammonia formed from the new decomposing diatoms was at once oxidized to nitrite. Finally, the nitrite disappeared from all cultures in the usual way, appearing quantitatively as nitrate.

A somewhat different result was obtained in subculture 42G, which was prepared by adding new organic matter to a portion of 42B when the latter was approaching the end of the nitrite stage. As before, ammonia remained low throughout the whole time. During the first week nitrite disappeared exactly as in the mother-culture from which it had been prepared (42B), but after this it increased again, reaching a new maximum ten days later. Apparently the nitrite-forming flora was on the decline when this subculture was begun but was able to recover under the influence of the newly-formed products of decomposition. Nitrate formation seems to have occurred throughout this subseries. It is probable that we have here a case in which ammonia-, nitrite- and nitrate-forming floras were active at the same time.

In the cultures so far described living diatoms were used as a source of new organic matter; consequently, vigorous ammonia formation was not actually under way at the start of each subculture. In the next two cultures organic matter was introduced which was already in the ammonia-formation stage. Fresh diatoms were added to a fresh quantity of harbor water (No. 42D). After 12 days in the dark, when ammonia was being formed rapidly, portions of this culture were withdrawn and mixed with equal amounts of older cultures in various stages of the decomposition cycle. Thus, subculture 42E consisted of an equal mixture of 42D and 42C, the latter taken when the nitrite had reached its maximum. In this case the ammonia introduced with culture 42D disappeared rapidly, with a corresponding rise in nitrite. Evidently the nitrite-forming flora of culture 42C was still active when the new, partially-decomposed organic matter was added.

Subculture 42F was prepared by separating a portion of the original culture, No. 42, when nitrite had begun to diminish, and adding an equal amount of 42D containing organic matter in the stage of ammonia formation. Both ammonia and nitrite disappeared rapidly, in contrast to the last preceding subculture, 42E, probably due to the fact that the nitrate-forming flora in the parent culture was at that time the most potent one.

The last culture, 42H, behaved in a somewhat similar manner. This consisted of a portion of culture 42A, separated at a time when the nitrite was about half converted to nitrate. To this was added a large number of partially decomposed diatoms, centrifuged from a culture which had stood for six days in the dark. A relatively small increase in ammonia was observed during the first days, with a subsequent rapid decrease.

Nitrite was present somewhat longer than in 42A, but never reached a very high level. It is likely that during this whole time nitrate formation proceeded rapidly.

The following conclusions may be drawn from the study of culture 42 and its subcultures: Ammonia formation does not interfere with the formation of either nitrite or nitrate, in such concentrations as we observed. The strict sequence of processes in our normal decomposition experiments can therefore hardly be due to any inhibiting action of ammonia or other initial products of decomposition upon nitrite or nitrate formation. More likely is it connected with a very slow development of the oxidizing floras. Doubtless, however, some other, hitherto unrecognized factor must also be involved. A slow increase in the nitrate-forming population, for example, is alone insufficient to explain why it should require weeks, or even months, for the first traces of nitrate to appear, whereas once the process has started the nitrate maximum may be reached in five days.

These observations are not necessarily inconsistent with experiments we have described previously, involving deep sea water, in which there was evidence of a retarding influence on the development of the oxidizing floras. This influence has not yet been explained, but seems to be connected with some unknown special property of the deep sea water used.

The course which the decomposition will take, when new organic material is added, will depend upon the flora which predominates. In general, a shortening of the cycle will occur, as far as the newly added material is concerned. The original culture, No. 42, took 55 days to complete its cycle. In the various subcultures the mean time from the addition of new organic matter to the end of the cycle was 36 days, or 41 days if one includes the initial period of decomposition of the added organic matter before its addition to Series 42E, F and H.

DURATION OF THE CYCLE

As pointed out in previous papers, the time required for the decomposition cycle varies considerably in different series. It seemed possible that the initial concentration of organic matter might be a determining factor in this connection and Series 47 to 50 were set up to investigate this point. The four cultures contained amounts of particulate nitrogen varying from 185 γ to 768 γ per liter. As shown in Fig. 2, this factor seems to be of some, though not of very great importance. The rate of disappearance of particulate nitrogen was nearly the same in each case. In the higher concentrations the ammonia maxi-

num was reached a little earlier and nitrite appeared and disappeared more rapidly. The total time for the cycle varied from 61 days in the highest concentration to 88 days in the lowest.

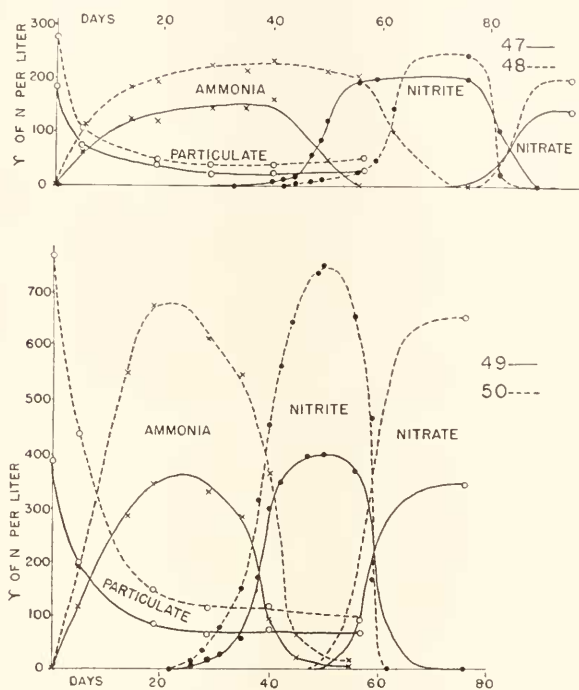


FIG. 2. Series 47 to 50. Influence of varying concentration of particulate matter. Filtered sea water with different amounts of *Nitzschia Closterium* added. Decomposition in the dark. Time in days. Different forms of nitrogen in micrograms (gamma) per liter.

The nature of the suspended organic matter also determines the duration of the cycle, as the next series show. Series 43 and 45 (Figs. 3 and 4) were set up with the same harbor water; in 43 was suspended a small amount of mixed plankton, in 45 a large amount of yeast. In No. 43 the nitrogen cycle proceeded in the normal way, but in No. 45 nitrite appeared only very slowly, with no formation of nitrate when the experiment was terminated after $5\frac{1}{2}$ months.

It has been shown that the length of the decomposition cycle depends upon the source of the water and it has been suggested that this might involve the action of growth-promoting substances upon the bacterial flora. Series 44 and 46 (Figs. 3 and 4) were planned as an approach to this question. Two samples of harbor water, the same as in Nos. 43

and 45, were evaporated to dryness and the salt residues ignited at 600–700° C. for 5 hours, to destroy organic matter. The salts were dissolved in the original volume of distilled water, with a little HCl, and the pH brought back to between 7.5 and 8.2 with NaOH. To the two (Nos. 44 and 46) were added amounts of mixed plankton and yeast, respectively, corresponding to the quantities in Series 43 and 45. In

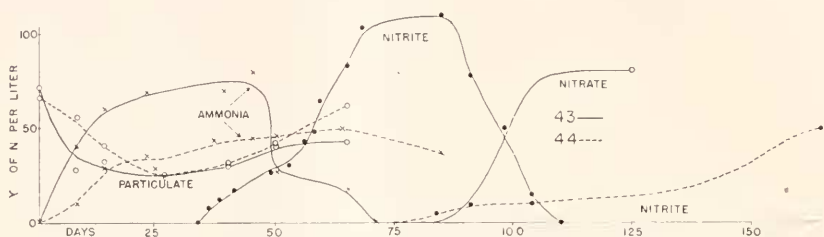


FIG. 3. Series 43 and 44. Mixed plankton added to filtered sea water (No. 43) and to a "synthetic" water made by redissolving the ignited salt residue of evaporated sea water (No. 44). Time in days. Different forms of nitrogen in micrograms (γ) per liter.

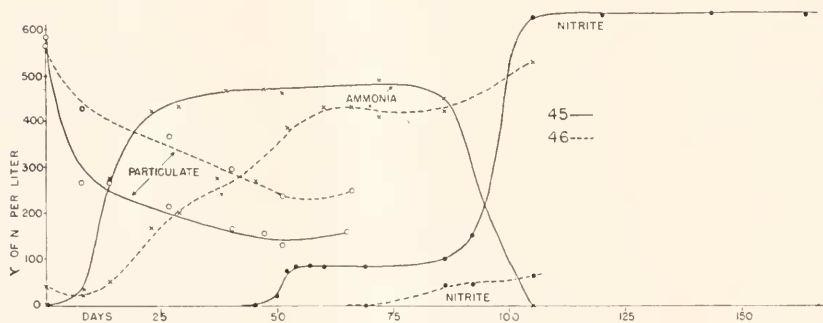


FIG. 4. Series 45 and 46. Yeast added to filtered sea water (No. 45) and to a "synthetic" water made by redissolving the ignited salt residue of evaporated sea water (No. 46). Time in days. Different forms of nitrogen in micrograms (γ) per liter.

both 44 and 46 the formation of ammonia was much slower than in the untreated water of Series 43 and 45, an effect even more pronounced on the formation of nitrite, which had not reached its maximum at the termination of the experiments. This apparently indicates that some "growth-promoting factor" had been eliminated from the water by the process of ignition, but further investigation will be necessary before a definite conclusion can be reached.

SUMMARY

1. With a recurrent supply of particulate organic matter, the formation of ammonia, nitrite and nitrate may take place simultaneously. The process which predominates will depend upon the stage at which the new organic matter is introduced.

2. The nature of the suspended particulate matter is of considerable importance in determining the total duration of the decomposition cycle, but the level of its original concentration is only a minor determining factor.

3. There is some evidence of a "growth-promoting" factor, normally effective in the decomposition cycle, but which can be destroyed by high temperature.

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